

Patent
825466-100140

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

Claim 1 (currently amended). A method of transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological features of a neuronal cell, comprising:

(a) culturing an epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin;

(b) transfecting said epidermal basal cell, in vitro, with one or more eukaryotic expression vector(s) containing at least one cDNA encoding a human neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, such that at least one of the neurogenic transcription factor(s) is expressed in said cell;

(c) growing the transfected cell in the presence of at least one antisense oligonucleotide comprising a segment of a human MSX1 gene and/or human HES1 gene, ~~or to a of either of these~~, in an amount sufficient to suppress the expression of functional MSX1 gene product and/or HES1 gene product; and

(d) growing said epidermal cell with a retinoid and at least one signal molecule selected from the group consisting of CNTF, sonic hedgehog, sonic hedgehog aminoterminal peptide, and IL-6, whereby the cell is transdifferentiated into a cell having one or more morphological, physiological and/or immunological feature(s) of a neuronal cell.

Claim 2 (previously amended). The method of Claim 1, wherein the eukaryotic expression vector(s) of the transfection step comprise a CMV promoter sequence operatively linked to a DNA(s) encoding the neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, and wherein the DNA

Patent
825466-100140

sequence encoding the neurogenic transcription factor is of human origin.

Claims 3-4 (cancelled).

Claim 5 (previously amended). A transdifferentiated mammalian cell having one or more morphological, physiological and/or immunological feature(s) of an astroglial cell, comprising:

a cultured epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cell transfected with one or more expression vectors comprising a CMV promoter sequence operatively linked to a DNA(s) encoding a neurogenic transcription factor NeuroD1, NeuroD2, ASH1, Zic1, Zic3, or MyT1, wherein the DNA sequence encoding the neurogenic transcription factor is of human origin, said cell being treated with at least one antisense oligonucleotide comprising a segment of a human MSX1 gene or a human HES1 gene and wherein said cell was grown in the presence of a retinoid and at least one signal molecule selected from the group consisting of CNTF, IL-6, sonic hedgehog, and sonic hedgehog aminoterminal peptide, thereby transdifferentiating said epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of an astroglial cell.

Claims 6-7 (cancelled).

Claim 8 (original). A transdifferentiated cell produced by the process of Claim 1.

Claims 9-10 (cancelled).

Claim 11 (currently amended). A kit for converting, in vitro, epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cells into cells having one or more morphological, physiological and/or immunological feature(s) of a neuronal or astroglial cell, said kit comprising:

(A) one or more eukaryotic expression vector(s) containing cDNA encoding a human neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1,

Patent
825466-100140

Zic1, Zic3, and MyT1, ~~or a non-human counterpart of any of these;~~

(B) at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of the human MSX1 gene, the human HES1 gene; and

(C) a retinoid and at least one signal molecule selected from the group consisting of CNTF, sonic hedgehog, and sonic hedgehog aminoterminal peptide.

Claim 12 (original). The kit of Claim 11, further comprising instructions for using (A), (B), and (C) in transdifferentiating a mammalian subject's epidermal basal cell(s).

Claims 13-15 (cancelled).

Claim 16 (previously amended). The transdifferentiated cell of Claim 8, wherein the cell further displays the physiological feature of a lack of mitotic activity under cell culture conditions which induce differentiation in neural progenitor cells.

Claims 17-21 (cancelled).

Claim 22 (previously amended). The transdifferentiated cell of Claim 8, wherein the cell is of human origin.

Claim 23 (previously amended). The transdifferentiated cell of Claim 8, wherein the transdifferentiated cell has a morphological, physiological, or immunological feature specific to an astroglial or oligodendroglial cell.

Claim 24 (cancelled).

Claim 25 (previously amended). An in vitro cell culture derived from the transdifferentiated cell of Claim 8, comprising a plurality of cells that express one or more morphological, physiological and/or immunological feature(s) of a ~~glial~~ neuronal cell.

Claim 26 (previously amended). The method of Claim 1, wherein culturing a the proliferating epidermal basal cell from a proliferating epidermal basal cell population derived

Patent
825466-100140

from a patient's skin cell population comprising one or more epidermal basal cell(s) comprises separating basal cells from keratinocytes using a calcium-free medium.

Claim 27 (original). The method of Claim 1, wherein said antisense oligonucleotide(s) is modified with one or more thio groups.

Claim 28 (currently amended). A method of transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological features of a neuronal cell, comprising:

(a) replace with culturing an epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin;

(b) transfecting said epidermal basal cell, in vitro, with one or more eukaryotic expression vector(s) containing at least one cDNA encoding a human neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, such that at least one of the neurogenic transcription factor(s) is expressed in said cell;

(c) growing the transfected cell in the presence of at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of a human MSX1 gene and/or a human HES1 gene in an amount sufficient to suppress the expression of functional MSX1 gene product and/or HES1 gene product; and

(d) growing said epidermal cell with a retinoid and at least one signal molecule selected from the group consisting of CNTF, sonic hedgehog, sonic hedgehog aminoterminal peptide, and IL-6, whereby the cell is transdifferentiated into a cell having one or more morphological, physiological and/or immunological features of a neuronal cell.

Claim 29 (previously amended). The method of Claim 28, wherein the eukaryotic expression vector(s) of the transfection step comprise a CMV promoter sequence operatively

Patent
825466-100140

linked to a DNA(s) encoding the neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, and wherein the DNA sequence encoding the neurogenic transcription factor is of human origin.

Claim 30 (currently amended). A transdifferentiated mammalian cell having one or more morphological, physiological and/or immunological feature(s) of an astroglial cell, comprising:

a cultured epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cell transfected with one or more expression vectors comprising a CMV promoter sequence operatively linked to a DNA(s) encoding a neurogenic transcription factor NeuroD1, NeuroD2, ASH1, Zic1, Zic3, or MyT1, wherein the DNA sequence encoding the neurogenic transcription factor is of human origin, said cell being treated with at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of a human MSX1 gene or a human HES1 gene, or to both, and wherein said cell was grown in the presence of a retinoid and at least one signal molecule selected from the group consisting of CNTF, IL-6, sonic hedgehog, and sonic hedgehog aminoterminal peptide, thereby transdifferentiating said epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of an astroglial cell.

Claim 31 (previously amended). A transdifferentiated cell produced by the process of Claim 28.

Claim 32 (currently amended). A kit for converting, in vitro, epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cells into cells having one or more morphological, physiological and/or immunological feature(s) of a neuronal or astroglial cell, said kit comprising:

(A) one or more eukaryotic expression vector(s) containing cDNA encoding a human

Patent
825466-100140

neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1;

(B) at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of a human MSX1 gene or a human HES1 gene, or both; and

(C) a retinoid and at least one signal molecule selected from the group consisting of CNTF, sonic hedgehog, and sonic hedgehog aminoterminal peptide.

Claim 33 (original). The kit of Claim 32, further comprising instructions for using (A), (B), and (C) in transdifferentiating a mammalian subject's epidermal basal cell(s).

Claim 34 (original). The transdifferentiated cell of Claim 30, wherein the cell further displays the physiological feature of a lack of mitotic activity under cell culture conditions which induce differentiation in neural progenitor cells.

Claim 35 (original). The transdifferentiated cell of Claim 30, wherein the cell is of human origin.

Claim 36 (original). The transdifferentiated cell of Claim 30, wherein the transdifferentiated cell has a morphological, physiological, or immunological feature specific to an astroglial or oligodendroglial cell.

Claim 37 (cancelled).

Claim 38 (currently amended). An in vitro cell culture derived from the transdifferentiated cell of Claim 30, comprising a plurality of cells that express one or more morphological, physiological and/or immunological feature(s) of ~~glial~~ an astroglial cell.

Claim 39 (previously amended). The method of Claim 28, wherein culturing the proliferating epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cell population comprising one or more epidermal basal cell(s) comprises

Patent
825466-100140

separating basal cells from keratinocytes using a calcium-free medium.

Claim 40 (original). The method of Claim 28, wherein said antisense oligonucleotide(s) is modified with one or more thio groups.

Claim 41 (currently amended). A method of transdifferentiating an epidermal basal cell into a cell having one or more morphological, physiological and/or immunological features of a neuronal cell, comprising:

(a) replace with culturing an epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin;

(b) transfecting said epidermal basal cell, in vitro, with one or more eukaryotic expression vector(s) containing at least one cDNA encoding a human neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1, such that at least one of the neurogenic transcription factor(s) is expressed in said cell;

(c) growing the transfected cell in the presence of at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of a human MSX1 gene and/or a human HES1 gene in an amount sufficient to suppress the expression of functional MSX1 gene product and/or HES1 gene product; and

(d) growing said epidermal cell with a retinoid and at least one signal molecule selected from the group consisting of CNTF, sonic hedgehog, sonic hedgehog aminoterminal peptide, and IL-6, whereby the cell is transdifferentiated into a cell having one or more morphological, physiological and/or immunological features of a neuronal cell.

Claim 42 (original). A transdifferentiated cell produced by the process of Claim 41.

Claim 43 (currently amended). A transdifferentiated human cell having one or more morphological, physiological and/or immunological feature(s) of an astroglial cell, comprising:

Patent
825466-100140

a cultured epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cell transfected with one or more expression vectors comprising a CMV promoter sequence operatively linked to a DNA(s) encoding a neurogenic transcription factor NeuroD1, NeuroD2, ASH1, Zic1, Zic3, or MyT1, wherein the DNA sequence encoding the neurogenic transcription factor is of human origin, said cell being treated with at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of a human MSX1 gene or a human HES1 gene, or to both, and wherein said cell was grown in the presence of a retinoid and at least one signal molecule selected from the group consisting of CNTF, IL-6, sonic hedgehog, and sonic hedgehog aminoterminal peptide, thereby transdifferentiating said epidermal basal cell into a cell having one or more morphological, physiological and/or immunological feature(s) of an astroglial cell.

Claim 44 (original). The transdifferentiated cell of Claim 43, wherein the at least one marker expressed by the cell is GFAP.

Claim 45 (cancelled).

Claim 46 (currently amended). A kit for converting, in vitro, epidermal basal cell from a proliferating epidermal basal cell population derived from a patient's skin cells into cells having one or more morphological, physiological and/or immunological feature(s) of a neuronal or astroglial cell, said kit comprising:

(A) one or more eukaryotic expression vector(s) containing cDNA encoding a human neurogenic transcription factor selected from the group consisting of NeuroD1, NeuroD2, ASH1, Zic1, Zic3, and MyT1;

(B) at least one antisense oligonucleotide ~~corresponding to~~ comprising a segment of a human MSX1 gene or a human HES1 gene, or both; and

Patent
825466-100140

(C) a retinoid and at least one signal molecule selected from the group consisting of
CNTF, sonic hedgehog, and sonic hedgehog aminoterminal peptide.